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Histological And Histochemical Studies After Partial Hepatoectomy In Liver And Spleen Of Rats

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Abstract

Introduction :- The aim of this investigation is to study the effect of partial hepatoectomy on the histology and histochemistry of liver and spleen of male rats.

Material And Methods:- Twenty four rats were used in the present study and were divided into four groups"six rats each".group(I) and classified into four groups: Negative control group (I). 6 rats and group (II) hepatoectomized rats were given sub. Cut 0.1 ml vitamin K 3days consequently before surgical hepatectomy was performed. Histopathological examination of liver and spleen and frozen sections for alkaline phosphatase demonstration were undertaken in the 1st day , 7th day and 15th day following the surgical operation.

Results:- The histopathological results were manifested in hepatoectomized rats as congested portal vein, vacuolation and pyknosis in liver cells, fibrosis inflammatory cells infiltration around hepatic vein and increasing kupffer cells. In splenic tissue the histopathological changes were in the form of ill defined pulps with marked congestion and necrotic nuclei. After 15th days of operation marked recovery in both white and red pulps were recorded. The histochemical examination of alkaline phsophatase revealed marked attenuation after 1th and 7th days of operation in both liver and spleen of hepatoectomized rats followed by regeneration after 15th day of operation in comparison to the control group.

Keywords: Hepatic., Spleen, Liver, ALP.

Introduction

It is well known that a substantial increase in fibrosis usually leads to potentially lethal cirrhosis of the liver in humans (Ulieno *et al*, 2003) Although the pathogenesis of liver fibrosis is not quite clear, there is no doubt that the reactive oxygen species (ROS) play an important role in the pathological changes particularly in cases of liver toxicity and radiation exposure (Maisin, 1998). On the other hand, many investigators have been studied the restoration of the liver rat following partial hepatectomy (Higgins *et al*, 1931; Hakcova et al 1996 and Kropacova et al., 1998.)

Nagino *et al*, (1999) correlated serum alkaline phoephatase (ALP) levels with liver function and its regeneration after major hepatoectomy. They concluded that serum ALP levels can be an indicator of liver function after hepatectomy, but it doesn't reflect morphological regeneration of the liver.

However, Osada and Saji, (2004) reported the clinical significance of monitoring alkaline phosphates level to estimate postoperative liver failure after hepatectom

Material And Methods

Drug:

0.1 ml viatmine K (anticoagulant) was injected subcutaneously for 3 days consecutive days before surgical operation. according to Ainley and James ,(1977)

Animals:

In this study, 24 male albino rats (100 – 120gm) were used. The animals were housed in cages under good ventilation , adequate standard diet and free supply of water.

The animals were divided into:

Group I: Negative control group consisted of 6 rats.

Group II: III&IV;Each group consisted of 6 rats. Animals were injected with 0.1 ml vitamin "K" 3 days before surgical operation, 2/3 partial hepatoectomy(from the left lope)was performed according to Higgins et al, (1931) and Hakova et al, (1996), Animals were anesthesized with ether,dissected and small fresh piece of liver ,transverse section of spleen were obtained after $1^{st(gr\ 1)}$, $7^{th(gr11)}$, and $15^{th(gr1V)}$ days following the operation. Tissues were fixed in neutral formal saline, dehydrated in ascending grades of alcohol, cleared in xylol,and embedded in paraffin wax. Samples were serially sectioned at a thickness of 5-6 μ. The sections were then stained using haematoxyline and eosin. Frozen sections were cut at 5-6μ and stained with Gomori's stain to demonstrate the alkaline phosphates enzyme activity according to Pearse, (1975).

Results:

Liver:

Examination of liver sections from control groups revealed normal histological structure of hepatic tissue Fig (1-1). Histopathological changes of liver after 2/3 partially hepatoedomy revealed congested hepatic portal vein, hypertrophied hepatocytes. in presence of kupffer cells after 1st day of treatment were noticed. Fig. (1-2).

Mean while, 7th day post-treatment proved distortion of the normal architecture of hepatocytes, widening of central vein, fatty degeneration besides some nuclei appeared pyknotic and others karyolysis were detected (Fig 1-3).

After 15th day of the treatment, showed massive number of kupffer cells appeared in the spaces between the blood sinusoids with vacuolated cytoplasm were observed (Fig. 1-4).

Spleen:

Histological examination of spleen sections from control showed the normal histoloical structure of spleen from white and red pulp. The white pulp consists of lymphoid follicles while the red pulp consists of different blood elements (Fig 2-5 on the other hand microscopic examination of spleen 2/3 partial hepatoectomy on 1st day of treatment showed internal splenic hemorrhage, ill defined pulps since larg areas are destructed (Fig. 2-6).

Histological sections from 2/3 partially hepatoectomy rats revealed distorted splenic architecture with necrotic nuclei and others karyolysis after 7th days of treatment (Fig 2-7).

However, spleen sections of hepatoectomrized rats after 15th days of treatment illustrated marked recovery both in white and red pulp were detected (Fig 2-8).

Histochemical studies in rat liver:

Alkaline phosphatase demonstration:

The present data in liver hepatocytes revealed moderate dark brown granules stain of alkaline phosphates activity in control rats (Fig 3-9).

Regarding the present histochemical data revealed marked decrease in phosphatase activity after 1st and 7th days of treatment Located in the parenchyma of liver tissues were detected (fig 3-10, 11) Later, after 15th days of treatment, revealed moderate distrbution of alkaline phosphates activity were noticed (fig 3-12).

Histochemical studies in rat spleen:

Alkaline phosphates activity:

The histochemical findings revealed negative white pulp for alkaline phosphates stain in control rats, whereas positive in red pulp alkaline phosphates activity were detected. (fig 4 – 13).

The present data showed a decrease in alkaline phosphates activity of rat in splenic red pulps after 1st and 7th days of treatment were detected (fig 4-14, 15) finally, modeate reaction in phosphates activity post 15th days of treatment were observed (Fig 4-16).

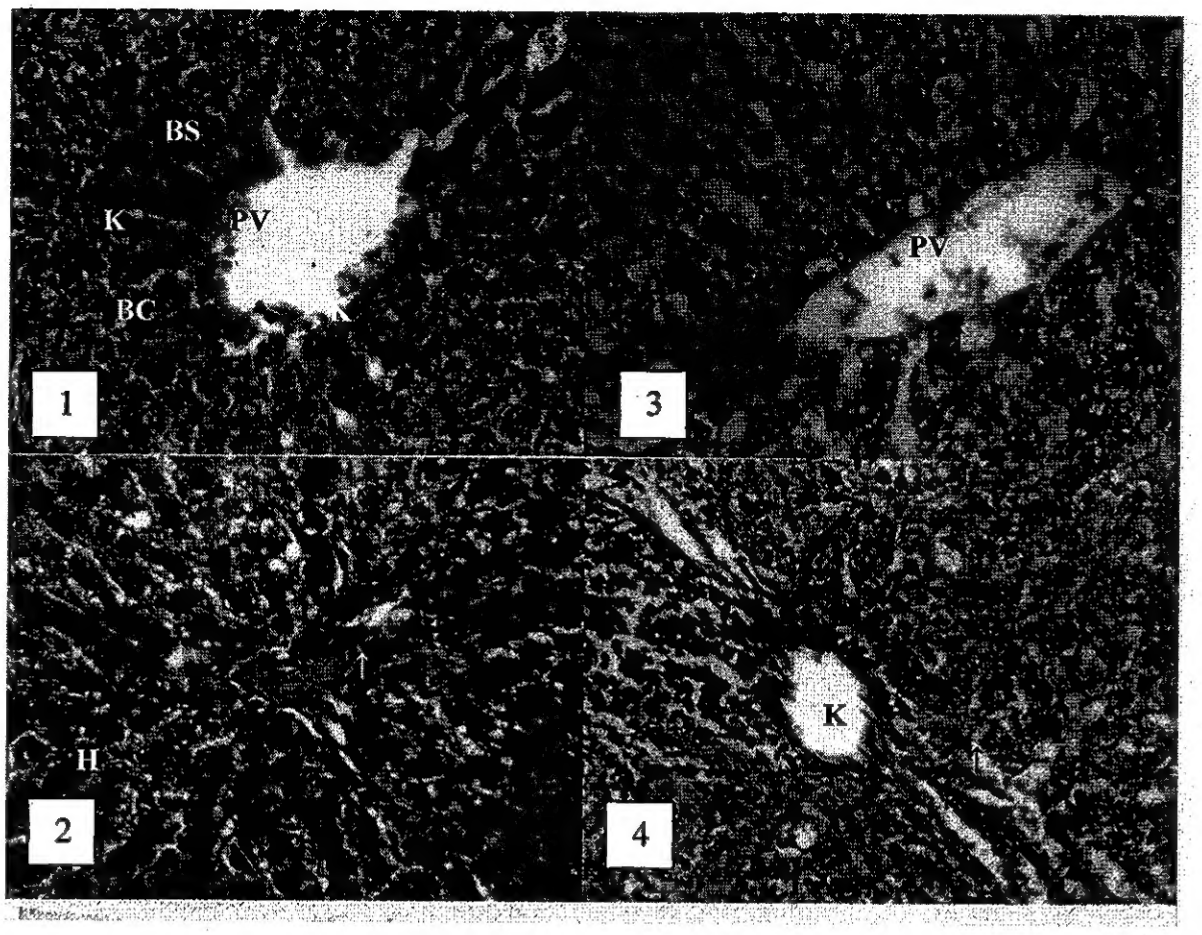

Fig. (1) Photomicrograph of sections in liver of control and experimental rat group (II).

1 – Photomicrograph of a section in liver of control rat showing normal histological structure, portal vein (PV), hepatocytes (H), kupffer cells(K), blood sinusoids (BS) and bile canalculi (Bc) (Hx & E x 250).

2 - Photomicrograph of a section in liver of group II after 1st day of treatment showing: congested hepatic portal vein (↑), hypertrophied hepatocytes obstruct the sinusoidal spaces in between (H) (Hx & E x 250).

3 - Photomicrograph of a section in liver of group II after 7th day of treatment showing dilatation of portal vein (PV), fatty degeneration (↑↑) in presence of vacuolated cytoplasm (↑) (Hx & E x 250).

4 - Photomicrograph of a section in liver of group II after 15th days of treatment showing marked increase of kuffer cells (K) and vacuolated hepatocytic cytoplasm (↑) (Hx & E x 250).

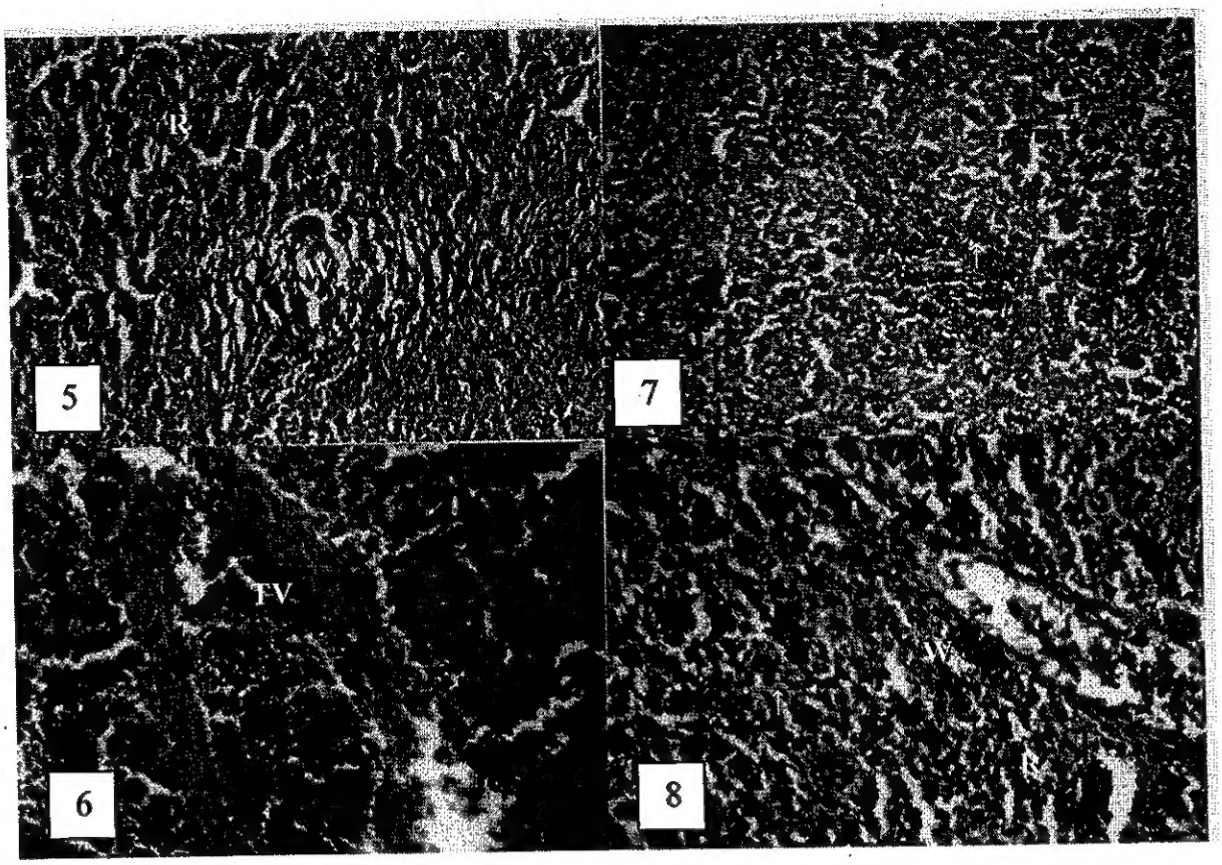

Fig. (2) Photomicrograph of a sections in spleen of control rat and experimental rat group (II).

5 - Photomicrograph of a section in spleen of control rat showing the normal histological structure of the white and red pulps (W & R), central arteriole (↑) (Hx & E x 250).

6 - Photomicrograph of a section in the spleen of group II after 1st day of partial hepatoectomy showing marked congestion of trabecular vessels (TV) with prominent internal hemorrhage (↑) (Hx & E x 400).

7 - Photomicrograph of a section in the spleen after 7th day of treatment, showing loss of its normal architecture and shrinkage white pulp (↑) (Hx & E x 250).

8 - Photomicrograph of a section in the spleen after 15th day of treatment, showing pram put recovery both white and red pulps (W.R) in slight lymphocyte infiltration (↑↑) (Hx & E x 400).

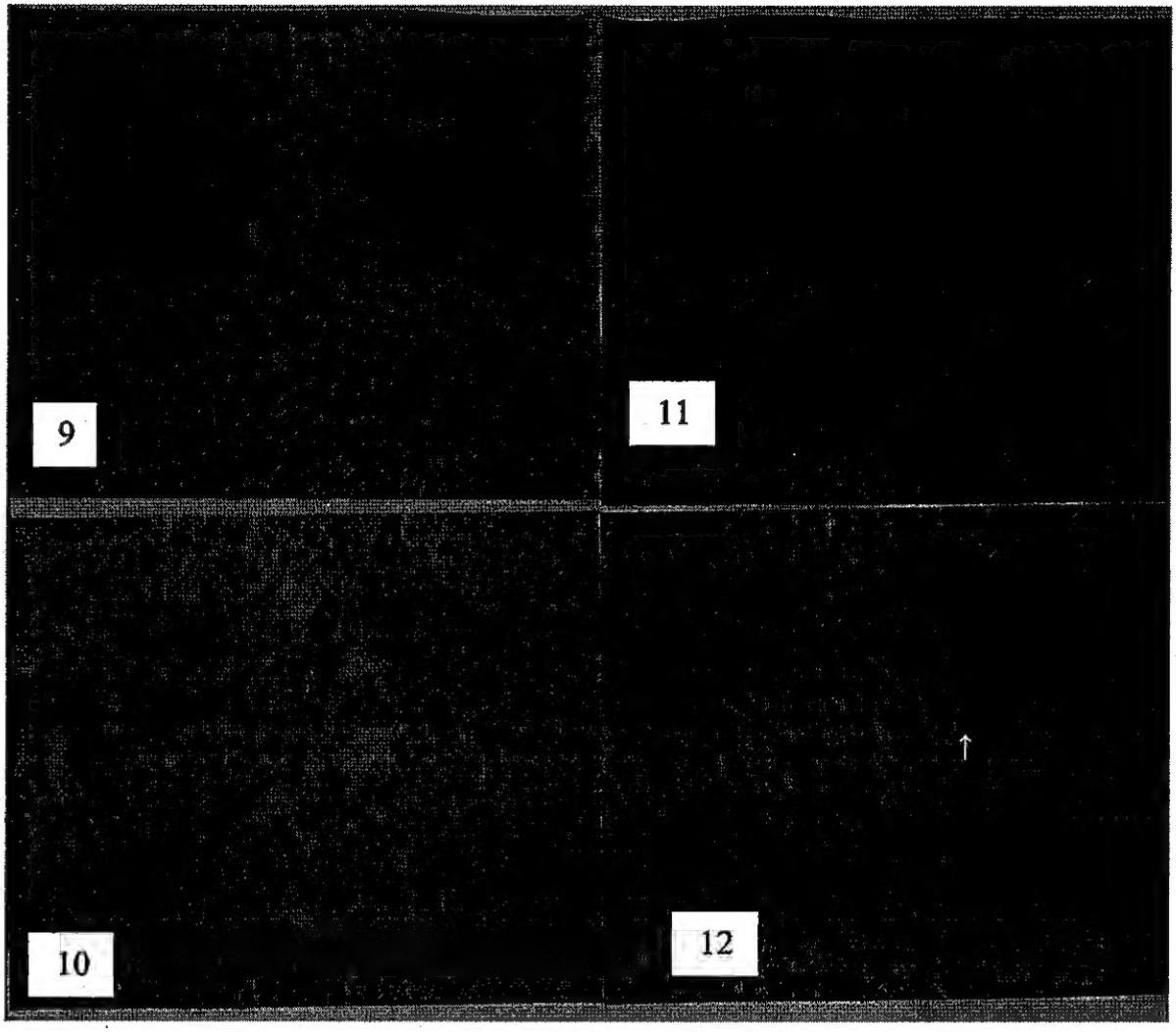

Fig. (3): Photomicrograph of a section in the rat liver and experimental rat group (II).

9 - Photomicrograph of a section in liver of control rat showing moderate distribution of alkaline phosphatase activity as dark brown granules (↑) (Gomori's x 400).

10- Photomicrograph of a section in liver of group (II) after 1st day of treatment showing decrease phosphatase activity in most of hepatocytes (↓) (Gomori's x 250).

11 - Photomicrograph of a section in liver of group (II) after 7th days of treatment showing decrease alkaline phosphatase (↓) (Gomori's x250)

12 - Photomicrograph of a section in liver of group (II) after 15th days of treatment showing moderate alkaline phosphatase activity (↑) (Gomori's x250).

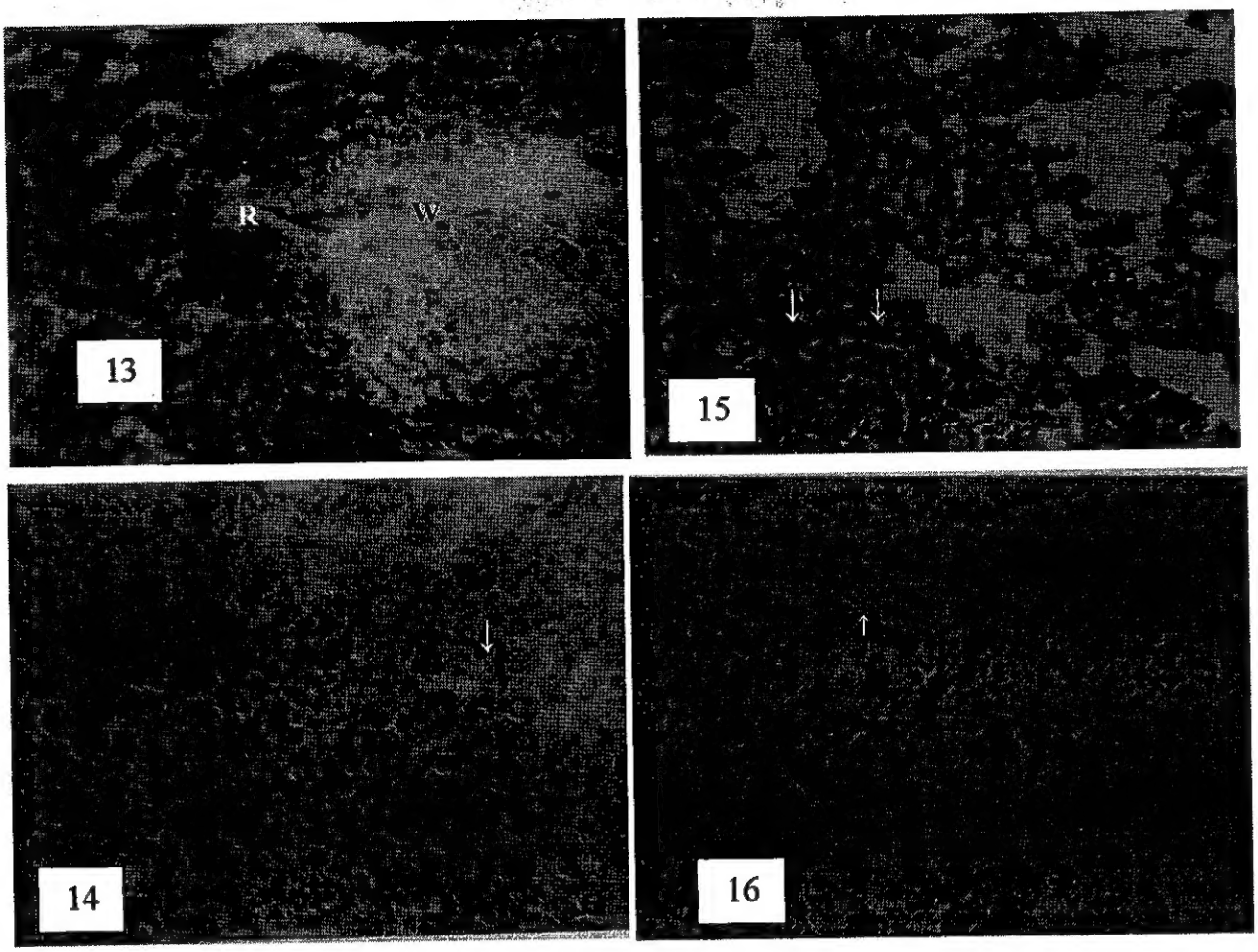

Fig (4): Photomicrograph of a section in the rat spleen and experimental rat group (II)

13 - Photomicrograph of a section in spleen of control rat showing negative white pulp (W) and positive red pulp phosphatase activity (R)(Gomori's x 400)

14 - Photomicrograph of a section in spleen of group (II) after 1st day of treatment showing decrease alkaline phosphatase activity (↓)(Gomori's x 400)

15 - Photomicrograph of a section in spleen of group (II) after 7th days of treatment showing decrease in phosphatase activity (↓) (Gomori's x 400)

16 - Photomicrograph of a section in spleen of group (II) after 15th day of treatment showing moderate in phosphatase activity (↑) (Gomori's x 400).

Discussion

The problem of liver regeneration has come into focus during the past several decades. Many histological studies and recently a growing number of biochemical and histochemical investigations have been devoted to this important problem

In the present study, histological and histochemical parameters were applied in the liver and spleen of rats after 2/3 partial hepatoectomy to detect its effects at varying intervals of time on 1st, 7th and 15th days post-surgical operation.

These findings could be attributed to the regenerative process started on first post operation day by the compensation hypertrophy followed by increasing mitotic activity of hepatocytes with the maximum on the 30 hours after partial hepatoectomy.

Moreover,the histopathological data showed changes of spleen sections after 1st and 7th day of partial hepatoectomized rats including congestion of trabecular vessels, shrinkage of white pulp with loss of normal architecture, and widening of blood vessels with lymphocytic infiltration followed by marked recovery after 15th day of treatment were detected. In fact, these results could explain an increase in the process of lipid peroxidation and a decrease in the activity of the antioxidant enzymes of the body with the consequent damage of cellular biomembranes El-Habit *et al.*, (2000) and Saad *et al* (2001).

On the other hand , alkaline phosphatase enzyme is a membrane bound glycoprotein is used as a marker enzyme for the plasma membrane.

Among these,the data indicated a decrease of Alkaline phosphatase in the liver and spleen red pulp after 1st and 7th day post hepatectomy. The decrease in alkaline phosphatase activity is similar to different pathological conditions in response to various stimuli Knolwes *et al*, (1979), In starvation Moussa *et al* (1987), in case of mercury intoxification Sastry and Rao (1981), in insecticide treatment Mousa *et al.*, (1987) and antibiotic treatment El Beih *et al* (1993), (1998) After radiation exposure Omaima *et al*, (2001). On the other hand, Melen *et al.*, (1985) who studied the effect of lysosomal enzymes in plasma, Liver and spleen from rats with carbon tetrachloride induced liver cirrhosis and reported no consistent increase in plasma, spleen or liver lysosomal enzyme activates.It is concluded from the present data marked improvement of alkaline phosphatase in hepatectomized rats after 15th days of treatment in both liver and spleen tissue section. These results could be beneficial when planning for radiotherapy programme in cancer patients,treatment of liver disease of different itiology including toxic hepatitis,antilipid peroxidative and liver degeneration.

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دراسات هستولوجية وهستوكيمائية في كبد وطحال الجرذان بعد النزع الجزئي للكبد

د. نبيلة عبد الرحمن عبد المتعال

قسم بحوث بيولوجيا الأشعاع بالمركز القومي لبحوث وتكنولوجيا الأشعاع

يهدف هذا البحث إلى دراسة تأثير النزع الجزئي للكبد هستولوجيا وهستوكيمائيا على كبد وطحال الجرذان.. وقد استخدم عدد 24 من الذكور وقسمت إلى مجموعتين حيث أعتبرت المجموعة الأولى الضابطه السالبة والمجموعة الثانية اعيد تقسيمها إلى ثلاث مجموعات للدراسة بعد اليوم الأول اليوم السابع واليوم الخامس عشر من حقنها تحت الجلد أو فيتامين K لإيقاف النزيف ثلاث أيام قبل العمل الجراحي .. تم التخدير والحصول على عينات الكبد و الطحال في الأيام المذكورة، وكما تم الحصول على عينات مجمدة للتعرف على محتوى الفوسفاتيز القاعدي لجميع المجاميع ومقارنتها بالمجاميع الضابطه.

أوضحت نتائج الفحص النسيجي للكبد والطحال بعد العمل الجراحي حدوث تغيرات واضحة من احتقان دموي للوريد الكبدي مع انتفاخ في الخلايا الكبدية وظهور نزيف واضح للطحال وفقدان الهيكل النباتي للطحال في اليومين الأول والسابع من المعاملة . بينما اوضحت القطاعات النسيجية للكبد استعادة نشاط الخلايا الكبدية وكثرة خلايا كوفر في الفراغات الموجودة بين الجيوب الدموية وبالمناظر أظهرت استعادة الهيكل النباتي لكل اللب الأبيض والأحمر مع وجود الخلايا الالتهابية في الأوعيه الحوجزية لطحال الفئران المنزوعه الكبد.

كما أظهرت النتائج نقص في نشاط الفوستاتيز القاعدي بعد اليوم الأول والسابع من العمل الجراحي في خلايا الكبد والطحال وإحداث تفاعل متوسط لمحتوى الفوستاتيز القاعدي في كل نسيجي الكبد والطحال بالمقارنه بالمجاميع الضابطة.